BIOLOGICAL BULLETIN

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Marine Biological Laboratory

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BIOLOGICAL BULLETIN

THE OVIPOSITION, COCOONING AND HATCHING OF AN ARANEAD, THERIDIUM TEPIDARIORUM C. KOCH.[1]

THOS. H. MONTGOMERY, JR.

During the course of collecting a series of accurately timed stages of the eggs of one of our most familiar spiders, the *Theridium tepidariorum*, I had occasion to accumulate quite a number of observations upon the reproductive habits. Published accounts of such habits in spiders are very few, there is much still unknown, so that it seemed worth while to write up these notes since they are the most extensive yet made upon any single species.

In an earlier contribution[2] I presented an account of this species along with others, and described the moulting, copulation, sperm-induction by the male, cocooning and care of the young. I then described three cases of cocooning, and timed the sequence of cocoons for eight females.

The present observations were made at Woods Hole, Mass., from the fifth to the twenty-sixth of August of the past summer. An unusually large colony of these spiders was found upon an old stone wall in a wood, to which they had probably strayed from some buildings adjacent to one end of the wall. About the first of August the spiders were beginning to form their first cocoons, and upon each of most of the webs of females one or more males were present. Two or three weeks later most of the males had disappeared. My previous study showed that

[1] Contributions from the Zoölogical Laboratory of the University of Texas, No. 76.

[2] "Studies on the Habits of Spiders, particularly those of the Mating Period," *Proc. Acad. Nat. Sci. Philadelphia*, 1903.

there may be frequent copulations before the first act of oviposition, and even between successive acts. But since the males begin to disappear before the full series of cocoons is made, and especially because my captive females produced successive cocoons without the presence of males, and yet these eggs were fertile, it is apparent that copulation before the first act of oviposition suffices for the fertilization of the later sets of eggs. Nearly 150 females were kept in small cages consisting of wooden and paper boxes covered on one side by glass; there was very little mortality among them, and at the end of the month they were manumitted in good condition.

The processes of oviposition and cocooning were observed in part or completely in a large number of cases, and may be summarized as follows. The female commences the cocooning by biting through certain threads in a particular region of the web; she then gathers the cut ends together with her legs, thus making a free space in which to work and at the same time forming a composite thread that serves for the initial suspension of the cocoon as well as of herself during the process of construction. She spins upon the lower end of the suspension thread, and combs out the new line into a fluffy ball; this is the beginning of the base of the cocoon, and the spider employs for the combing mainly the third pair of legs, but to some extent the fourth also. From this point in the operations until the cocoon is nearly completed the spider hangs to lines of the surrounding web by her first and second pairs of legs, with her cephalothorax directed vertically and above the abdomen. When the fluffy fundament of the cocoon base has a diameter somewhat larger than the length of the cephalothorax of the spider, the latter rotates this loose textured ball with her palpi and third pair of legs, and with the fourth pair in alternate application draws a thread out from her spinnerets and applies it to the lower rim of the fluffy ball. She continues this motion until the base of the cocoon has attained the form of an inverted cup, the upper convexity of which is attached to the suspensory line. Towards the close of the base-making her spinning becomes gradually slower. Then she proceeds to oviposit. She lifts herself slightly by contracting the anterior pairs of legs, then

presses her genital aperture against the concavity of the base; a yellow drop of fluid pours out and immediately adheres to the concavity of the base, the eggs rapidly flowing out into it until the whole reaches a size quite equal to that of the spider's abdomen. The spider touches the surface of the egg mass only with the lower surface of her abdomen; during the oviposition there are repeated pressures of the abdomen against the egg mass. The yellow fluid is at first of thin consistency and soon dries upon the eggs; but in drying it does not glue them together. Shortly after the discharge of all the ripe eggs the spider by a few quick contractions detaches her epigynum from the surface of the egg mass, and immediately starts to make the cover of the cocoon, spinning first upon the exposed surface of the egg mass until the latter is covered, then upon the surface of the base also. From her spinnerets she draws out a continuous, compound thread with the fourth legs used alternately, applying this thread to the growing cocoon, while at the same time rotating the cocoon slowly with the third pair of legs (with the occasional help of the second), the spider still hanging to the web above the cocoon by the first pair. Thus the eggs become quickly hidden from sight, by the making of a closely knit cover composed of one continuous thread. Throughout the whole operation the spider keeps pressing the tips of her palpi against the cocoon, which gives her a knowledge of the progress of the progress of the work; there can be no question that she is guided entirely by the sense of touch, for during her labor she is is so placed as to be unable to see the cocoon. Towards the close of the cover making she spins closer, with the result that the exterior surface of the cocoon has the firmest texture. The spinning finished, she feels over the entire surface with her palpi. Rarely does she leave the cocoon hung upon the suspensory thread, usually she carries it higher up into the web and attaches it to the object that roofs the latter.

The finished cocoon varies from yellowish to dark brown in color, and large ones may reach a diameter of 10 mm. It is quite smooth externally, pyriform and usually pointed at the upper end where the suspensory thread was first attached.

More than two hundred cocoons were produced by my spiders,

of which 188 were marked as to the exact or approximate time of oviposition. On a few days I did not commence observations until 6 a. m., and in such cases estimated the time of oviposition of prior cocoons by the state of completion of the cover; but in the majority of cases the precise time of the end of the act of oviposition was determined. From the accumulated data the following results were obtained:

1. *Time of Day of Oviposition.* — This is probably always in the morning and usually in the early morning; in one case a cocoon was made some time between 11 a. m. and 4 p. m., while I was absent from the laboratory, and in this single case oviposition may have occurred later than the noon hour. The next latest case was one at 11.29 a. m. The following table shows the hours of oviposition and the number of cocoons formed in each:

Before 5	5–6	6–7	7–8	8–9	9–10	10–11	11–12
43	51	42	32	33	9	5	2

In the laboratory the spiders experienced about the same light conditions as they did upon the stone wall. Though my lamp was frequently burning at night in the room, and its light wakened the spiders to activity, yet it never induced them to start cocooning. The influence that occasions the cocooning can therefore not be one of amount of light, nor of sudden change from darkness to light. If it were amount of light they should be expected to cocoon at twilight as well as at dawn, yet they never do so. I do not think that cocooning is stimulated by any light condition, but that the stimulus to oviposition may be the oncoming of the warmth of the day after the coolness of the night. My records of cocoons of one and the same individual show that successive cocoons may be formed at different hours of the day, *i. e.*, that one individual does not maintain a particular cocooning hour.

2. *Time Duration of the Act of Oviposition.* — This was timed in 43 cases, the interval being measured from the moment of commencing extrusion of the eggs to the moment of commencing spinning of the cover. In one case it lasted just 2 minutes; in nine cases from 3 to 4 minutes; in twenty cases, from 4 to 5 minutes; in five cases, from 5 to 6 minutes; in six cases from

6 to 7 minutes; in one case, 8½ minutes and in one case, where the spider seemed feeble, 19 minutes. In the majority of cases it lasts from 3½ to 4½ minutes. There is remarkable uniformity in the length of duration of the act, which seems to be quite independent of the number of eggs discharged. After all the eggs have been extruded but while a point of the egg mass surface still adheres to the genital aperture, the spider may continue reiterated ineffectual efforts to discharge further eggs, and how long it continues to do so renders the duration of the process longer or shorter.

3. *Time Duration of the Spinning of the Base.* — This was timed for seventeen cases, showing a variation from 14 to 37 minutes; in eleven of these cases it was between 20 and 30 minutes.

4. *Time Duration of the Spinning of the Cover.* — This was observed in thirty-two cases. In one case the process lasted 27 minutes; in five cases, 40 to 49 minutes; in eleven cases, 50 to 59 minutes; in two cases, 60 to 69 minutes; in three cases, 70 to 79 minutes; in four cases, 80 to 89 minutes; in three cases, 90 to 99 minutes; in one case, 110 minutes; and in one case where the spider lacked the left fourth leg, for 142 minutes. There is, accordingly, considerable variation in the duration of this operation, due not at all to the size of the egg mass, apparently also not to the rate of spinning, but rather to the thickness and firmness of the cocoon which varies greatly. The cover making is usually uninterrupted, but sometimes the spider may pause, then evidently from weariness. It may be that high nourishment would allow the greatest amount of silk secretion, and that in well fed individuals the cocoons be largest and take the most time in the making.

5. *Abnormalities in Cocooning.* — Abnormal cocoons are not rare with wild individuals, and relatively more numerous with my captive specimens. Generally an abnormal cocoon is due to a defect in the spinning of the base; the latter may be too small or too loose, and the spider in working upon it is then liable to pull it out into an irregular form. Again, the issuing egg mass may by accident adhere to a leg of the spider, or even a portion of it become detached from the rest; when this happens the spider

may cease to spin, seemingly being cognizant of something out of the ordinary course. Or the cover may be incompletely made, leaving the egg mass partially exposed, this is apparently due to lack of sufficient space for the spinning operations. Once a mistake is made the spider appears to be unable to rectify it, a fact that I have remarked in my earlier observations (*l. c.*); the whole process appears to be strictly instinctive and the spider seems unable to modify it more than quantitatively, and unable to learn by experience. An individual may make an imperfect cocoon, then construct a following one quite perfect; or the reverse may happen. When the cocoon is particularly irregular, as when the egg mass becomes more or less broken, the spider may either eat the eggs, or may detach them from the web and allow them to fall to the ground.

6. *Time Intervals between Successive Cocoons Formed by the same Individual.* — Of 113 timed intervals between successive cocoons raised in my cages, there was one interval of 2 days, 2 of 3 days, 13 of 4 days, 26 of 5 days, 18 of 6 days, 19 of 7 days, 12 of 8 days, 10 of 9 days, 3 of 10 days, 4 of 11 days, 4 of 12 days, and 1 of 13 days. More than half of the intervals ranged from 5 to 7 days. In all probability the rapidity in the rate of the succession of cocoons depends upon the degree of nourishment of the spider, because some that I purposely starved furnished no cocoons at all. My captives were of course not as well fed as they would have been in the wild state, and though I kept them fairly well supplied with young grasshoppers and locustids this by no means equalled their natural diet in either variety or amount. It is probable that these spiders in a natural state, with normal feeding, would furnish on the average cocoons at intervals of from four to six days. The shortest time in which a succession of 4 cocoons was made by any one of my spiders was 14 days, comprising one interval of 4 days and two of 5 days. The largest number of cocoons I have found in any wild web was eight.

7. *Hatching of the Young.* — Thirty-nine cocoons were kept to determine the time of hatching of the young. Of these, 3 failed to hatch; 3 hatched after an interval of 11 days; 3, of 13 days; 18, of 14 days; 2, of 15 days; 9, of 16 days; and 1, of

17 days. The majority, accordingly, hatched at intervals of from 14 to 16 days. Different generations of cocoons do not have different rates of hatching. Cocoons made on the same day need not hatch at the same time; thus of 8 cocoons made on August 20, 3 hatched in 11 days, 2 in 13 days, 2 in 14 days, 1 in 16 days; and of 11 cocoons made on the morning of August 26, 9 hatched in 14 days, and 2 in 16 days. All these cocoons were removed from the web, placed in separate glass vials, and kept together under the same conditions of light, temperature and moisture. The differences in the rate of hatching are probably due to the difficulties experienced by the young in emerging, for where the cocoon is thinnest the hatching is earliest. Most of the spiderlings emerge in the early morning, but they may come out in the late morning and the afternoon. Each of the few most vigorous spiderlings makes a small circular aperture through the wall of the cocoon, and through these few openings all the rest find their way. The young are at first decidedly positively heliotropic, and it is the light shining into the first made exits that probably guides the less precocious individuals out of the cocoon. The weakest may not emerge until several hours or even days after the most vigorous. In this species, unlike the lycosids, I have found no evidence that the mother aids the young to hatch; for I removed from the webs a number of cocoons immediately after their completion, thus precluding any opening of the cocoon by the mother, yet all of the young made their way out. The mother exhibits some degree of bravery in guarding the cocoon, especially when the latter is newly formed, though never to the extent of allowing herself to be injured, and if she is roughly handled she invariably drops from the web.

8. *Protective Value of the Cocoon.* — I removed eggs, at various intervals after oviposition, from cocoons and placed them in flat glass dishes in the ordinary diffuse light of the laboratory; in all these cases normal spiderlings resulted. Therefore the presence of a cocoon is not necessary to normal development and, further, its value is probably not to exclude the light. One batch of eggs was opened onto a dish of water, where they floated; after a number of days they become covered by a mould that killed

them. Probably the main value of the cocoon is to protect the eggs from enemies, for were the eggs not enclosed they would fall singly or in a mass to the ground, become removed from the guardianship of the mother, and be subjected to a great variety of rapacious foes.

9. *Relation of the Cocoon to the other Forms of Aranead Architecture.* — On this important question I will dwell only briefly at this point, reserving a fuller discussion for later. McCook[1] concludes: "The spinning work of spiders may be classified generally as, first, the Snare, spun for the capture of prey; second, the Enswathment, by which insects are disarmed and prepared for food; third, the Gossamer, used for purposes of aqueous and aerial locomotion; fourth, the Cocoon, spun for the propagation and protection of the species; and fifth, the Nest, which is a domicile more or less elaborate and permanent within and under which the aranead dwells for protection against the exigencies of weather and the assaults of enemies." Menge[2] had previously called attention to the web that the males of certain species spin for the deposition of their sperm, preparatory to charging their palpi with it; this has also been described by me (*l. c.*), and it may be called the "sperm-web." Then Wagner[3] in a most important analysis of the architecture of spiders directed especially towards its phylogenetic significance, has distinguished the web spun by some forms for protection during the moult; and then divides what McCook calls the "Nest" into: "*La retraite*-construction, destinée pour *la demeure de l'araignée*, où elle passe tout son temps et qu'elle ne quitte que pour la chasse. A l'epoque de la ponte cette retraite peut servir de loge au cocon." And into "*Le nid*-construction, destinée au séjour de la femelle et à contenir le cocon." As far as I comprehend this distinction of Wagner's, the "retraite" is a nest made by both sexes, the "nid" one built by the female only. It seems to me questionable whether this distinction of Wagner's is a valid one, and McCook's term "nest" had best be retained without especial subdivision, though surely different kinds of nests may be distinguished.

[1] "American Spiders and their Spinning Work," Vol. 1, Philadelphia, 1889.

[2] "Ueber die Lebensweise der Arachniden," *Neueste Schr. naturf. Ges. Danzig*, 4, 1843.

[3] "L'Industrie des Araneina," *Mém. Acad. Sci. St. Pétersbourg* (VII.), T. 42.

Therefore we have to enumerate: the *cocoon*, *snare*, *nest*, *parachute* (a somewhat more definite term to replace McCook's "gossamer"), *sperm-web*, *enswathment* and *moult-web*. Of these constructions the cocoon occurs in all species, and probably the nest also; these may then be said to be the most conservative or most persistent kinds of architecture in spiders. It is probable that the nest or protective habitation was the first construction to be elaborated, perhaps first merely some cranny that later became lined with silk by the spider; and that the spider laid its eggs first without lining against the wall of this nest, then later spun upon them a cover of silk. This is of course merely an inference because we know nothing of the ancestors of the modern spiders. But the cocoon, by which is meant the immediate silken envelope of the eggs, may in this way be explained as a derivation of the nest of which it formed at first an integral part; the original base of the cocoon would have been the silken investure of the nest, the cover of the cocoon a later addition. If this suggestion be correct, then the phylogeny of the cocooning would have been as follows: (1) oviposition against the wall of the nest; (2) the addition of a cover to the eggs; (3) a special base spun upon the wall of the nest, the eggs upon which received a cover (a condition realized by certain attids, clubionids and drassids); (4) the formation of a cocoon with base and cover apart from the nest.

Wagner has distinguished two kinds of cocoons: those made of two pieces, and those of a single piece, a distinction based upon the appearance of the completed structure. I would maintain, on the contrary, that it is probable that in all modern araneads the cocoon is spun of two pieces, first a base, then a cover, for I have observed (*l. c.*) this process in lycosids, agalenids, dictynids, theridiids, epeirids and drassids. One may not judge from the form of the finished cocoon as to its mode of construction, and, so far as I know, in all cases where the process has been followed a base is first spun, the eggs oviposited upon it, then a cover made. There seems to be no positive evidence that the cocoon is ever made in a single piece. One of the best diagnostic characters of Araneads is the making by the mother of a silken covering, composed of two pieces, for the protective investment of the eggs.

10. *Parasitic Hymenoptera in the Eggs of Theridium.* — In none of the cocoons raised by my captive spiders were there any parasites, but about one out of every thirty or forty wild cocoons was found to be infected with a tiny hymenopteron, black in color, the males winged and the females wingless, of a length somewhat less than the diameter of an egg of the spider. Prof. Wm. H. Ashmead has kindly identified these as Proctrotrypids, constituting a new species (*montgomeryi*) of the genus *Bæus*. The males hatch some hours or a day in advance of the females, and die soon after the momentary copulation with the latter. The wasps generally hatch out from the cocoons at about the same time the spiders hatch from the uninfected eggs.

Three cocoons, from four to six hours old, were placed in a dish containing wasps that had hatched from other cocoons one or two days previously. The female wasps walked over the surface of the cocoons, continually tapping them with their antennæ; most of the females oviposited into the cover of the cocoons, or at least frequently projected their ovipositors into them. A smaller number made their way into the interior of the cocoons, taking about ten minutes to bore, mainly by rotation of the large and sharp-rimmed head, a small circular aperture into the cocoon. After several of the female wasps had entered in this way I cautiously opened a cocoon and under the compound microscope could observe the wasps piercing the spider eggs with their long, needle-like ovipositors; they seem not to succeed in piercing every egg that they attempt. A single wasp inoculates in this way a considerable number of spider eggs. Only one wasp emerges from any one spider egg, so it is probable that the wasp places only one egg in a spider egg, but I hope to decide this point by a careful study of the infected eggs. Rarely if ever are all the eggs in a cocoon so infected; the infected ones after three or four days turn a dull brown color. If an infected cocoon is kept until hatching in a closed vial, so that neither the emerging spiders nor the hatching wasps can escape, the spiderlings that have escaped infection ultimately catch and eat the adult wasps.

REGENERATION OF CERTAIN STRUCTURES IN FUNDULUS HETEROCLITUS.

C. V. MORRILL, JR.

It is a fact of common knowledge to all observers of fish kept in aquaria, that portions of the fins, lost through accident, or purposely removed will be replaced by new structures which cannot be distinguished, in many cases, from normal fins. This ability to regenerate has been examined by Duncker (1905), Morgan (1900, 1902), Nussbaum and Sidoriak (1900), Mazza (1890) and Broussonet (1786). The following experiments were made to test the effect of cutting off the fins close to the body. The regenerative powers of the operculum, the lower jaw, the scales and the lens were also tested.

The various sets of experiments described below were tried on different fish, no one fish having more than one fin removed, except in the case of pectorals and pelvics which were removed from the same fish, the right pectoral and left pelvic being always selected for convenience.

I wish to express my thanks to Prof. T. H. Morgan, who kindly suggested this problem and under whose direction it was completed. My thanks are also due to the staff of the New York Aquarium, and to Dr. F. B. Sumner, director of the U. S. Fisheries Laboratory at Wood's Hole, Mass., who kindly placed the necessary tanks and fish at my disposal.

DORSAL FIN.

On October 10, 1905, eighteen fish had the dorsal fin cut off as close to the body as possible. These were examined from time to time until December 29, 1905, when due to various causes, principally fungal growth, the number had become reduced to thirteen. All of these with one exception were regenerating a new fin. The excepted case was kept under observation three months longer but at the end of that time, twenty-six weeks after operation, it had developed only a small

pointed knob in the middle of the old scar. The remaining twelve were not kept after December 29 (twelve weeks after operation). In all of these the regeneration was proceeding rapidly at this time, some having the new fin half completed.

On February 16, 1906, a second set of twenty-five fish had the dorsal fin cut off close to the body. These regenerated more rapidly than the first set and in five weeks' time, twenty-one of these which still remained, were regenerating along the entire length of the cut surface, in many cases the new growth being one eighth of an inch in height. Three weeks later, fifteen only were still living; the increase in height of the fins was distinctly visible in this short time, though more so in some individuals than in others. Three weeks later (at the time of writing) and twenty-three weeks after operation, fourteen were still alive. The new fins in some cases were regenerating evenly and possessed the normal number of fin rays (eleven). But in other cases, the new growth appeared along part of the cut surface only. An examination of the anatomy of the fin and the exact direction of the cut reveals the cause of this irregularity. Usually in trimming the fin close to the body, the dermal fin-rays are severed a short distance distal to their articulation with the bony supporting rays, beneath the surface. Sometimes, however, if the flesh at the sides of the fin is pressed down by the scissors and the fin at the same time held firmly, the cut will pass, in some places, through the enlarged proximal part of the dermal fin rays and regeneration in consequence will be much slower, or may not take place at all in parts so affected. The cut may even at times remove the dermal rays completely. These facts, I think, will account for cases in which regeneration is irregular, *i. e.*, when the new structure does not contain the normal number of fin-rays. Thus complete or nearly complete removal of the fin-rays prevents regeneration of the fin.

Anal Fin.

The anal fins of eighteen fish were cut off close to the body on October 25, 1905. The wounds healed up rather quickly but showed no signs of regeneration until two months later, when of the thirteen fish remaining, five showed slight signs of

new growth. Six weeks later the regeneration was fairly started in five fish which still survived. These five were examined one week later, *i. e.*, sixteen weeks after removal of the fin and it was found that three showed slight regeneration while the other two had a new fin about half the size of the original one. In these last, the fin was normal in shape and position, though small, and the rays parallel to one another as in the old fin. Ten weeks later, twenty-one weeks after removal of original fin, the regeneration was almost complete, the new fin having the normal number of fin-rays and being scarcely distinguishable from ordinary anal fins.

Here as in the case of the dorsal fin, the cut usually passes a short distance distal to the articulation of the dermal with the bony fin rays. But it may pass a little further in through the articulations if not carefully made, as the body is soft in the region of this fin. A cut of this kind results, as might be expected, in no regeneration. The cut may, however, pass through the articulations of some rays and the shafts of others; then the new growth takes place from the cut ends of the latter only, producing an irregular structure with less than the normal number of fin-rays.

The external prolongation of the oviduct in the females, which passes down the anterior end of the fin is, of course, severed with the fin. It regenerates, as might be expected, only when the anterior fin-ray which supports it, regenerates, not otherwise.

Caudal Fin.

On October 5, 1905, the caudal fins were removed from eighteen fish, the cut being made in a curve so as to remove the entire fin, without at the same time cutting off any of the tail vertebræ proper. This operation is rather severe; there is usually some bleeding and the edges do not close over the wound very readily. In consequence the number of fish lost is usually considerable. After twelve weeks, five fish only remained, all showing regeneration of the fin to some extent. Two weeks later the regeneration was still proceeding slowly but somewhat irregularly, the new fin-rays being bent downward, forming a considerable angle with the original horizontal direc-

tion of the rays, and the rays themselves were fewer in number than in normal fins. This result was due to very close cutting; entire rays were removed in some places, and in others, where stumps remained an alteration in direction of these stumps occurred in some way when the wound healed over. In one case the new fin grew straight out in the normal direction but at different rates of growth in different parts so that it presented a finger-like appearance. This abnormality was probably due to a jagged cut, the fin-rays being disarticulated in some places while in others, the stumps were left in situ. In still another fish no regeneration took place at all although the wound healed over smoothly. This fish was kept under observation four months. Here the cut probably either disarticulated the rays or left too small a stump for regrowth to take place.

On January 9, 1906, the tails of twenty-four fish were cut off in such a way as to remove a portion of the body of the fish, including several vertebræ. Obviously this operation was extremely severe. These fish when returned to water were unable to assume a horizontal position but remained head downward at the top of the tank. Three days later eleven were still living. These latter had succeeded finally in reaching the bottom of the tank and some were swimming about in a nearly normal position. At this time, January 12, another set of twenty-four fish, cut as described above, were put into the same tank with the eleven just mentioned. Three days later only seventeen were left of the entire number, thirty-five. This number gradually decreased until the entire lot died within a month's time.

Another set of twenty-four were cut in the same way but it was found impossible to keep them alive. The wounds remained open and the flesh rotted away from the sides of the vertebræ.

In the above sets, various sizes of fish were used. In still another set ten *small* fish were employed and kept in shallow dishes in the laboratory in order that they might assume a horizontal position. Within a month all of these had died with one exception. This might have been due to unfavorable conditions in part, though I have kept fish living in these shallow dishes over three months. In the exception noted, the wound had healed over smoothly and a very small white knob of tissue ap-

peared over the cut end of the spinal cord. This fish was kept under observation for three months but during this time the white knob did not increase perceptibly in size. As far as external examination could determine no regeneration of bone took place.

Pectoral and Pelvic Fins.

On October 25, 1905, twenty-four fish had their right pectoral and left pelvic fins removed. The cuts were made as close to the body as possible. In three months all died but three. These showed no regeneration of the pectoral fin but in one case a slight indication of a regenerating pelvic. Two weeks later two of these were still living, both showing the regenerating pelvics well under way and a well-defined indication of regenerating pectorals. Unfortunately the two fish died a few days later. Another set of twelve fish were cut in the same way but all died within a month, showing no signs of regeneration.

A third set of twenty-five fish cut as above, were started on February 16, 1906. Nine weeks later eighteen were still living; five of these showed regenerating pelvics and one, an indication of regenerating pectoral; the rest no regeneration of either fin. Two weeks later (at the time of writing) seventeen were living. Two of these showed regenerating pectorals distinctly, in the shape of a small knob of tissue which no doubt contained two or three regenerating rays, since the fish moved the structure to and fro like a fin. Eight of this same lot were regenerating their missing pelvic fins, the new structure in some cases being one eighth of an inch long.

An examination of the direction of the cut in the removal of the pectoral fins shows that in most cases all the dermal fin rays are removed entire, *i. e.*, disarticulated. Occasionally, however, the cut does not completely remove the uppermost one or two rays. These rays extend inward a little further than the others and so their proximal stumps may remain in situ and give rise to little pointed structures as described above, but never to a complete fin. In the case of the pelvic fins, it is not possible as a rule to cut them so close in; thus it more frequently happens that stumps of the rays are left, sometimes a few, sometimes all. Regeneration takes place from the cut ends of these only.

From the foregoing facts, the lack of regeneration of the paired fins in so many cases is seen to be due to complete removal of the dermal fin rays. These results fall in line with those described above for the unpaired fins.

Operculum.

On January 15, 1906, a set of six fish had a piece of the operculum removed, in order to determine whether the actual bony structures of the fish would regenerate. The piece removed was about one eighth of an inch broad and half an inch long, and was taken from the lower posterior border. Six weeks later two of these fish remained, but showed no signs of regeneration. A few days later, these also died. One fish with operculum cut as above was kept in a dish in the laboratory for four months, but at the end of this time showed no signs of regeneration.

The same experiment was tried with two sets of fifteen fish each at Wood's Hole. These fish were kept from June 20, 1906, to August 25, 1906, but at the end of this period showed no signs of regeneration.

Fish operated on in the above manner are sometimes difficult to keep alive since the gills are at first exposed to attacks of parasites and the nibbling of other fish. In a short time, however, the branchiostegal membrane spreads over and partly closes the opening.

Lower Jaw.

On March 23, 1906, pieces about one quarter of an inch long, were removed from the right-hand side of the lower jaws of thirty-six fish. Six weeks later, twenty-eight were still living. The jaws had grown again to such an extent that some of them could scarcely be distinguished from normal ones. Whether new bone took the place of that removed, was not accurately determined, though microscopic examination of sections of three of these new structures seemed to indicate that cartilage at least had regenerated.

Attempts were also made to remove larger portions of the jaw, or the entire jaw, but these always resulted in the death of the fish even if the wound healed. This may have been due to

the difficulty the fish experienced in grasping and holding its food.

Scales.

On June 20, 1906, the scales were removed from definite areas of a number of fish. This operation was performed in such a way as to injure as little as possible the underlying dermis, each scale being separately pulled out with a pair of fine forceps. Three sets of fifteen fish each were used in this experiment, each set of fish having the scales removed from a different part of the body. In three weeks time the new scales were coming in rapidly, some of them being one third the normal size, though very thin and soft. Two weeks later, the new scales were still only one-half to three quarters the length of a fully grown scale, though many were as broad. It appears then, that regenerating scales grow faster in breadth than in length. At this period or a little earlier the characteristic dotted pigment appears. At first the dots are comparatively few in number but continue to increase until the pigmentation, normal, for the position of the scale is attained. Thus each scale, from whatever region it is taken, is replaced by a new one pigmented exactly like the original. The fish in this experiment were kept ten weeks in all and at the end of that period the regenerated scales were practically normal in size, thickness and color though sometimes slightly irregular in shape.

Lens.

On June 21, 1906, the lens was removed from one eye of a number of fish. The operation was performed by slitting the cornea with a sharp scalpel and then gently pressing the eyeball with forceps until the lens popped out. In all thirty-six fish were used which were divided into two sets of eighteen each. In one of these sets the iris was slit in addition. These fish were kept until August 29, about ten weeks, when eleven fish remained in one set and nine in the other. When the lens is squeezed out some of the vitreous humor goes with it and after the operation the eyeball appears flattened. However the cornea heals over very quickly and the eye soon assumes its regular contour, differing only from normal apparently, in a slight dilation of the pupil.

The lost vitreous humor is apparently made good in some way. An examination of the eye at the end of the experiment (after ten weeks) revealed not the slightest indication of a new lens. Regeneration of the lens in *Triton* and *Salamandra*, has been obtained by Colucci ('91), Wolff ('95) and Fischel ('98). These results cannot then be extended to the teleost, *Fundulus heteroclitus*. However, it still remains to be shown whether the regenerative powers of the eye are not better developed in other species of teleosts.

Summary and Conclusions.

The general conclusions to be drawn from the foregoing experiments are the following:

1. In *Fundulus heteroclitus* the dorsal, caudal, anal, pectoral and pelvic fins will regenerate even when cut off close to the body, provided the proximal ends of the dermal fin-rays remain in situ. No regeneration takes place except when the stumps are present, and of sufficient size.

2. The operculum does not regenerate.

3. The lower jaw completes itself if a piece is removed.

4. The scales, if carefully removed, are quickly replaced by others.

5. The eye does not regenerate a new lens.

The results on the regeneration of fins support in general Broussonet's earlier work and that of subsequent writers. The ability to regenerate a portion of the jaw has been found by Spallanzani (1768) and others in the salamander. The lack of ability to regenerate a new lens in *Fundulus*, however is peculiar, since the salamander, an animal of a higher order, possesses it to a marked degree. It would be interesting in this connection to test the regenerative powers of the eyes of elasmobranches and ganoids, as being more generalized types than the teleosts.

In regard to Broussonet's statement that those fins which are the most useful, regenerate the quickest, it seems more probable that the conditions of the cut, as described for each experiment above, are the factors which determine the rate of regeneration, given equal conditions of temperature, food, vitality, etc. Indeed it is difficult to estimate the relative rate of growth, for in any

one set of fish kept in the same tank and having the same fins removed, the differences in the rate of renewal are so great that some of the new fins may have completed half their growth when others are just beginning to show definite regeneration. Again, the caudal fin though obviously the most important in many fish does not regenerate, perceptibly, faster than the others. Finally, leaving aside the caudal it is difficult to say which fin is the most important. The observations which Osburn ('06) has recently made on the functions of fins seem to indicate that the pectorals are more useful than the dorsals and anals while my own results show that the former regenerate much slower. Broussonet was probably led to his statement concerning the relative rate of regeneration in different fins by his acceptance of the idea of regeneration as an adaptation. *A priori*, the caudal fin would regenerate, the most rapidly, on the hypothesis, since it is the most useful. The facts as I have tried to show, do not bear this out.

At this point the question naturally arises as to the relation between regeneration and liability to injury. It is true that the fins, extending out from the body as they do, are subjected to injury through brushing against obstructions, attacks of predatory animals, etc.; therefore, one might say, they acquired the power to regenerate as an adaptation to these conditions. But why, on the adaptation hypothesis, should the jaw regenerate so readily, when it is extremely improbable that this structure would ever be lost in a natural state? From the lack of regenerative power in the operculum and eye no conclusions can be drawn for or against this hypothesis, since these structures are not especially liable to injury under normal conditions. The power to regenerate scales, however, seems truly adaptive.

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October 6, 1906.

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NOTE ON THE GEOTROPISM OF ARBACIA LARVÆ.

E. P. LYON.

As soon as the blastulæ of the sea urchin begin to swim, they come to the top of the water. They remain in this locality during the blastula and gastrula stages but become scattered when they reach the pluteus condition.

The gathering at the top is not a light effect, for it takes place in total darkness.

The gathering is not due to lack of oxygen, for the larvæ go up in an inverted test-tube filled with sea water and remain at the top a long time. With the dense gathering of thousands of blastulæ at the top of such a tube the gradient of increasing oxygen supply must be from above downward, yet the larvæ do not go down. A narrow tube closed at one end was nearly filled with water containing young arbacia. An air bubble was introduced into the tube about the middle of its length. The tube was then placed with the closed end directed up. The larvæ went up in both ends of the tube, those above the bubble going directly away from the oxygen supply.

Experiments in tubes kept at constant temperature showed that the gathering was not due to currents in the water. Nor was the gathering due to the larvæ being lighter than sea water. Their specific gravity, on the contrary, is considerably higher, being about 1.060.

That the gathering of larvæ at the top is a true gravity effect is indicated by their moving against a centrifugal force. The best way to demonstrate this is by placing sea water containing many larvæ in rather long (say, 25 cm.), narrow tubes. On rotating these carefully, it will be found that beyond a certain radius (depending on the rate of rotation) the larvæ are precipitated, the acceleration in these parts of the tube being greater than they can swim against. Inside this critical circle the larvæ will be found to have moved toward the axis of rotation and to be gathered in dense crowds.

Paramœcia will rise to the top of a solution of the same density as themselves and in which they have, therefore, no weight. This fact, to my mind, is proof that gravity acts within the organism and not through the relative densities of water and organism. With the larvæ of arbacia, however, I was unable to get any definite gatherings in a solution of gum arabic and sea water of the same density as the larvæ. The viscosity of this medium is so great that such minute ciliated organisms as those under consideration can hardly move through it.

While convinced, therefore, that the gathering of the arbacia larvæ at the top of the water is a gravity effect, I am unable at the present time to say how gravity brings about the response. The possibility that the orientation is purely passive, a buoyancy effect, must be kept in mind, as well as the various theories proposed by Jensen and Davenport. However, on the basis of my paramœcium work, I consider it most probable that gravity acts directly on the cells.

The blastulæ from centrifugalized eggs have all the pigment on one side and are therefore presumably heavier on that side. Later examination shows that this pigment may be in any part of the gastrulæ or plutei. Such larvæ, although, as stated, presumably unbalanced, nevertheless come to the top of the water. I consider this observation opposed to the theory of orientation by buoyancy, for if the heavier portion were always directed down and this portion sustained no relation to the direction of locomotion, the organisms ought to be scattered through the water.

NOTE ON THE HELIOTROPISM OF PALÆMONETES LARVÆ.

E. P. LYON.

The striking peculiarity of the light reaction of these larvæ is that they orient themselves with the anterior end away from the light and then swim backward toward the light. If orientation alone is the criterion of a tropism, these organisms are negatively heliotropic. But they gather on the side of the disk toward the light and as ordinarily observed would surely be considered positive.

Blue light produces these effects as strongly as daylight. Red light is almost ineffective.

The orientation of the larvæ is usually extremely precise. The instant that light is admitted to a dish containing the larvæ, they all turn so that their heads are directed away from the source of light and the body axis is in the line of the rays. In many the abdomen is strongly extended so that the body axis is a straight line. These swim backward practically in a straight line. Very frequently they swim on their backs in a perfectly straight course to the light side of the dish. Others swim with ventral side directed down.

Besides the individuals which hold their bodies extended and exhibit very accurate orientation there are many which swim with the abdomen more or less flexed ventrally. These are inclined to rotate, but their general course is quite exact toward the light.

A study of the water currents, when India ink was added, showed that the backward movement was the result of strong forward beats of the swimmerets. The details have not been studied. The animals can move forward, but this form of locomotion is quite different from the backward type, being accomplished by a series of very rapid jumps or plunges. So quick were these forward movements that I could not accurately observe how they were accomplished. I believe that they were

due to strong extension of the tail, the animal first bending the body ventrally and then suddenly extending it. The jumping reaction occurred on strong stimulation of any kind : for example, on throwing light suddenly into the dish. The orientation with the head away from the light was accomplished by one or more leaps, but so accurately that one could hardly speak of trial and error. When the animals in swimming backward toward the light suddenly hit any obstruction, the springing reaction carried them momentarily away from the light. But they immediately began, under the influence of the swimmerets, to move backward toward the light. So it would often happen that on reaching the side of the vessel the larvæ would strike, jump away, swim back, jump away again, and so on many times without losing their orientation in the direction of the light rays.

It was therefore of some interest to know how these larvæ would behave if the sense of response to light could be reversed. I have made only a beginning of this study.

The effects of raising the temperature were not clear. For the most part the leaping reaction predominated. The animals darted rapidly about and lost their orientation to light.

Better results were obtained by diluting the sea water. If the larvæ were placed in a mixture of 50 c.c. distilled water and 100 c.c. sea water, a considerable proportion of them went to the negative side of the dish. This they did without changing their orientation. In other words they now employed the forward instead of the backward type of locomotion and thus arrived at the side of the dish away from the light. Often there seemed to be a conflict between the two methods of locomotion, the animals oscillating back and forth like shuttles, but always with the heads away from the light. Equal quantities of distilled and sea water gave even more pronounced results.

The responses were not always so regular and machine-like as I have described. Some of the more pronounced "negative" individuals moved by an irregular, mostly sidewise motion away from the light. Taken as a whole the "negative" effect secured by diluting the sea water was less precise and definite than the "positive" reaction. Also most of the "negative" individuals were only temporarily so.

On returning negative individuals to the undiluted sea water they at once swam to the light side of the dish, but without changing their orientation, *i. e.*, with head directed away from the light.

The question of nomenclature which these observations suggest may well await further investigations. The experiments here recorded were made at the Marine Biological Laboratory at Woods Hole.

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CHROMOSOME RELATIONS IN THE SPERMATOCYTES OF ONISCUS.

M. LOUISE NICHOLS.

Recent work on the germ cells of animals has shown that the chromosomes contained within one nucleus frequently are not all of the same size. It has been known for some time that in insects and spiders one or more of the chromosomes, the so-called accessory, is smaller than the others and also differs in its behavior. With regard to chromosomes other than the accessory, Sutton,[1] in his studies on the spermatogenesis of *Brachystola* discovered a well-marked difference in size. Montgomery's [2] studies of the chromosomes of various Hemipteran species also revealed a variation in size of chromosomes occupying the same nucleus, and McClung,[3] in a recent paper on the spermatocytes of Orthoptera has described a remarkable structure, strikingly larger than the other chromosomes, which he has named "multiple chromosome," and which he interprets as being formed by the association of a number of chromosomes. The still more recent work of Morgan [4] and Stevens [5] on aphids indicates much the same thing. Morgan studied the eggs of Phylloxerans and shows in his figures of *Phylloxera caryæ-globuli* a distinct difference in the size of the chromosomes. Miss Stevens investigated a number of species of plant lice and concludes that each species is characterized by a definite number of chromosomes.

Chromosomes of the same nucleus may differ not only in size but also in shape. In a paper published in 1902 [6] I showed that

[1] W. S. Sutton, "On the Morphology of the Chromosome Group in *Brachystola magna*," BIOL. BULL., 4, No. 1, 1902.

[2] T. H. Montgomery, Jr., "A Study of the Germ Cells in Metazoa," *Trans. Am. Phil. Soc.*, 20, 1901.

[3] C. E. McClung, "The Chromosome Complex of Orthopteran Spermatocytes," BIOL. BULL., 9, No. 5, 1905.

[4] T. H. Morgan, "Male and Female Eggs of Phylloxerans of the Hickories," BIOL. BULL., 10, No. 5, 1906.

[5] N. M. Stevens, "Germ Cells of Aphids," Carnegie Inst., May, 1906.

[6] M. L. Nichols, "Spermatogenesis of *Oniscus asellus*, Linn.," *Proc. Am. Phil. Soc.*, 41, No. 168, 1902.

in the equatorial plate of the first maturation division of the male reproductive cells of the wood-louse, *Oniscus*, there occur chromosomes of three kinds. Some are straight or dumb-bell shaped, others are crescent shaped. In these two forms the component parts of the bivalent chromosomes lie end to end. A third form exists in which the univalent chromosomes are joined not end to end, but side by side. In each type a split may be observed running the length of the chromosomes and the first division is therefore reducing. In the prophase the third type is represented usually by rings, occasionally by V-shaped structures (Figs. 7 and 14), while the crescents appear as curved rods and the remainder as straight or dumb-bell shaped rods. The reduced number of chromosomes, as far as could be determined, is sixteen. Of this number two have the ring form, two are crescents and the others are straight or dumb-bell shaped. The chromosomes vary somewhat in size, but in *Oniscus* the differences are not so great as in some of the insects before mentioned. The largest have the ring form, the smallest are straight (Figs. 1, 2, 4, 17, 18). Sometimes one of the two rings is complete or nearly so, the other incomplete (Figs. 1, 11). The rings lie on opposite sides of the nucleus, sometimes directly opposite, in other cases apparently shifted to one side or the other (Figs. 1, 2, 3, 8, 9, 11, 15, 16). Since they are the largest and most easily recognized of the nuclear elements they form convenient points of localization.

The crescent form is the next most easily recognizable. Of this type there are likewise two, one of them sometimes more strongly curved than the other. They also occupy opposite sides of the nucleus and at points between the positions occupied by the rings (Figs. 3, 4, 8, 9, 10, 11, 15, 16, 19).

The relative position of the rings and crescents is more readily determined than that of the rods, because they are not so numerous and are also distinguished by their shape. Repeatedly however I have observed an arrangement of three or four straight chromosomes in a row (Figs. 9, 12, 17). One of the smaller of the straight chromosomes, too, is often seen lying not far from one of the crescents (Figs. 8, 13, 17, 18).

These facts point to an individuality of the chromosomes and also indicate a tendency to localization in the nucleus. The evi-

dence for this is further strengthened by the manner in which the chromosomes develop from the resting nucleus. The resting nucleus is a network formed by the gradual lengthening and anastomosis of threads which retain their individuality until just before the formation of the nuclear membrane. From it arise the chromosomes of the first maturation division by a breaking apart of the threads of this network and their gradual shortening and thickening.

The question presents itself whether these relations of form and position of the chromosomes are peculiar to *Oniscus* or characteristic of the land isopods as a group. Two other genera I have examined, *Porcellio* and *Armadillo*. The spermatogenesis of these is similar to that of *Oniscus*, but the cells are smaller, in *Armadillo* so small as to make an examination of the chromosomes very difficult and uncertain.

The cells of *Porcellio* more nearly approach those of *Oniscus* in size and I was able to compare the prophase of the first division in the two animals. Although the chromosomes of *Porcellio* are smaller than those of *Oniscus*, the peculiarities of shape and arrangement are similar. In *Porcellio* also are present two rings lying opposite each other with a crescent between. More than one crescent in a nucleus, however, I have not seen. One at least of the straight chromosomes appears considerably smaller than the others and, as in *Oniscus*, frequently lies not far from the crescent (Figs. 5 and 6). I was not able to determine the number of chromosomes in *Porcellio*, as my preparations contained no spermatocytic mitoses. Besides the smaller size of the chromosomes would make an accurate determination even more difficult than in *Oniscus*. As far as can be judged from the appearance of the prophase, the number would seem not to be very different in the two genera.

The presence of chromosomes of the three forms described within the same germ nucleus is not then peculiar to *Oniscus*, but probably is characteristic of the group, the genera being visibly distinguished from each other by differences in size of the chromosomes and possibly by slight differences in the shape of homologous chromosomes.

Comparing the results obtained for various animals and plants,

it seems safe to conclude that any correlation existing between the shape of the chromosomes and the tangible bodily characteristics is not very close nor exact. Species quite distinct from each other may possess chromosomes of almost the same shape and size. Especially is this true of plant species. On the other hand, species closely resembling each other may have chromosomes of perceptibly different shape or size. This is even more obviously the case with the number of chromosomes. In some instances a difference in number may be accompanied by difference in sex,[1] in others by specific differences.[2] Again the same number may characterize groups as widely different as angiosperms, mammals and insects[3]; while related species of the same genus may differ in number.

Nevertheless in a general way there appears to be some correspondence between the bodily attributes of a group and the character and behavior of the chromosomes of that group. Chromosomes of a particular shape are more characteristic of certain groups than of others. Thus, the form in which the limbs of the bivalent chromosomes are twined about each other is frequently found in flowering plants and also in amphibia, the ring form is particularly apt to occur in arthropoda and a shape somewhat like a shepherd's crook, with a slender body and thickened, curved extremity, is often seen in mollusks and worms. Again the peculiar chromosome known as the accessory so far has not been found outside of the Tracheata.

No doubt the shape, possibly also the number of chromosomes, is determined by complex physical and chemical conditions which may not have any direct relation to visible bodily characters and two chromosomes of the same shape and size may contain potentialities of quite different nature, just as an apple and its waxen imitation, although of the same size and shape, have very diverse properties.

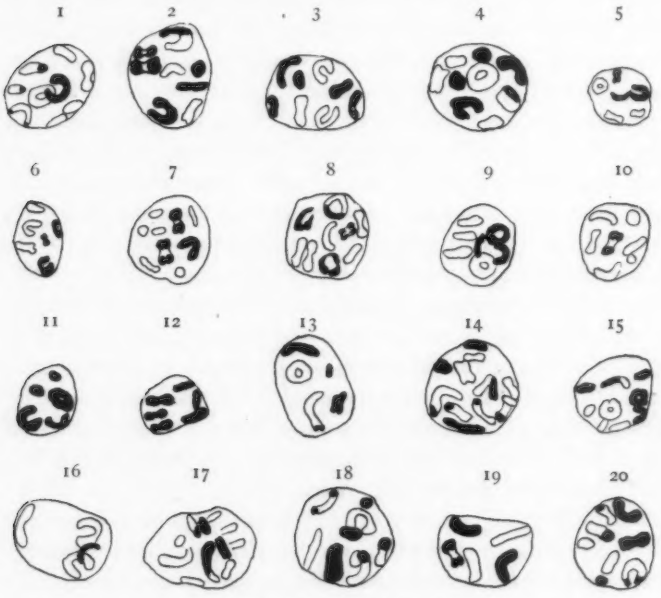
A parallel condition may be seen in the relation of the shape and size of the egg to the bodily characters of the organism developing from it. Forms differing as widely as fish and

[1] E. B. Wilson, "The Sexual Differences of the Chromosome Groups in Hemiptera," *Jour. Exp. Zool.*, III., No. 1, 1906.

[2] Montgomery, Morgan, Stevens, *loc. cit.*

[3] E. B. Wilson, "The Cell in Development and Inheritance," p. 206, 1900.

echinoderms may have eggs of about the same size and shape, but of varying potentialities. Yet here, too, there are peculiarities of constitution and behavior characteristic of certain groups rather than of others. There may be as good ground for determining the relationship of organisms by the behavior of chromosomes as by the character of cleavage in the egg, but in the former case the handicap is severe because of the minute size of the elements and the fact that changes of important nature may take place in the constitution of the chromosome without producing any effect tangible for modern microscopic methods. If, however, specific or other differences happen to coincide with visible variations in the chromosomes, such objects form convenient points of departure for the study of such problems as the determination of sex and the relation of paternal and maternal chromosomes in the cells of hybrid offspring.



EXPLANATION OF FIGURES.

With the exception of 5 and 6, the figures are prophases of the first maturation division of *Oniscus asellus* Linn. Nos. 5 and 6 are from *Porcellio*. The drawings were made with the camera lucida, Zeiss oc. 6, obj. $\frac{1}{12}$ imm. at the level of the table.

A PRELIMINARY NOTE ON ANT BEHAVIOR.

C. H. TURNER.

There is a wide-spread belief among living entomologists that ants slavishly follow the odor trail and Bethe insists that ants are mere reflex machines. For something over three years I have been conducting a series of experiments on the behavior of ants, the results of which do not harmonize with the above view. These experiments, which were performed upon a large number of southern species and a few northern species, show conclusively:

1. That the movements of worker ants are not controlled by tropisms.

2. That ants are not guided by a homing instinct.

3. That ants are enabled to take long trips by learning, by experience, the way and retaining what they have thus acquired. They learn the way in the same manner that vertebrates learn to open problem boxes and to run mazes.

4. That ants do not as slavishly follow the odor trail as is supposed.

5. That Sir John Lubbock was right when he said: "In determining their course ants are greatly influenced by the direction of the light."

6. The color of the pathway has little or no effect upon the home-going of ants.

7. That Dr. Wheeler is right in stressing the high development of the female; for the winged females often take part in the regular duties of the nest. I have had them learn the way home from new situations and assist the workers in carrying the pupæ home.

8. That the males are stupid and seem unable to solve even the simplest of problems. They seem to be more or less heliotropic.

9. The major workers of *Pheidole*, which Ernest Andre claims function as soldiers and do not take any active part in the ordinary work of the nest, frequently assist the workers in making

excavations; and, occasionally, assist in conveying pupæ from one place to another. I have never noticed one continue to carry pupæ for any considerable length of time.

10. Ants are affected by olfactory, optic, tactile, kinesthetic and probably auditory stimuli.

11. Ants seem to have fairly definite impressions of direction, both in horizontal and vertical planes, and of distance.

12. Ants have associative memory.

13. Such cases of division of labor as Romanes — quoting from Moggridge, Lespes, Belt and Herr Gredler — describes in his "Animal Intelligence," are to be looked upon as cases of incidental coincidence rather than as examples of mutual coöperation.

14. Ants can be trained to do certain simple things in the same way that vertebrates are trained.

15. In their activities ants display marked individual variations

In this preliminary paper, space forbids a discussion of the data upon which the above statements are based; that will be done in a paper which I expect to publish in the spring or summer of 1907. In this communication I merely desire to give some of the evidence that warrants the fourth and fifth statements.

In these experiments I used a card-board stage six inches square, from which a card-board incline, three fourths of an inch wide, led down to the island upon which the nest of the ants was confined. A great many pupæ and worker ants were placed on this stage. After many random movements the ants learned the way from the stage to the nest and back. The way once learned, the ants continued to work until all of the pupæ had been carried into the nest. I then replaced the ants and pupæ on the stage. The pupæ were again carried home. This was repeated over and over again; until, by their actions, I concluded that the ants were thoroughly acquainted with the way down the incline to the nest. Then a second incline was so placed as to lead from the opposite side of the stage to the Lubbock island. If, after a lapse of a few minutes, no workers descended this second incline, I concluded that the workers thoroughly knew the way down the other path. I then placed the first incline, which had

become scented by the foot-prints of the ants, in the place where the new incline had been and placed a new unscented incline in the place formerly occupied by the one the ants had been traversing. Thus there was an unscented path in the position of the old trail and the old familiar scented path was in a new position.

Now if ants are guided home solely by the sense of smell, they should either have spent approximately as much time learning the way down the new incline as they did before; or else, in their random movements, they should have happened upon the scented incline and gone down it. In reality, they did neither of these things. They went almost immediately down the unscented incline which occupied the position in space formerly filled by the incline down which they had been carrying pupæ to the nest.

Similar experiments, yielding the same results, were conducted with marked individual ants.

It will be seen at once that these experiments disprove not only Bethe's double polarized trail hypothesis but also Wasmann's assumption that their odor tracks have for ants an odor-shape which guides them home.

According to the current belief, ants going to and from my stages should have followed the same path. But experiments, conducted with marked individual ants, showed that this was not always the case. I found:

1. That the ant had to learn the way, not only from the stage to the island, but also from the nest back again to the stage and that it usually required more time to solve the second problem than it did to solve the first.

2. That sometimes an ant would regularly descend to the island along the top side of the incline and ascend to the stage along the under side of the same incline.

3. I have noticed a worker regularly descend from the stage by way of the incline and ascend the stage by way of its central support.

4. I have had ants ascend to the stage by way of one incline and descend by the other.

5. In passing from the nest to the foot of the incline the ant did not always follow the same path.

6. I have known an ant to regularly drop from the stage with a pupa and carry it to the nest and then return to the island, mount a section lifter, which I presented, and rest quietly thereon until it had been conveyed to the stage. Then it would step off, pick up a pupa and drop from the stage. This was repeated over and over again by the ant.

More than a quarter of a century ago Lubbock claimed that ants were influenced by the direction of the rays of light. These experiments have been either overlooked or ignored by recent continental writers. Although Sir John Lubbock's experiments were not so planned as to exclude the possibility of the effect noticed having been produced by heat or change in the intensity of light, yet it is hard to see any reason why they should have been so completely ignored by European writers. My experiments on the effect of light upon the home-going of ants, which were conducted under perfectly planned conditions of control, resemble very much certain of Lubbock's experiments.

Two kinds of tests were conducted: experiments with ants working in concert, and experiments with marked individual ants working alone.

In experiments of the first type a card-board stage was used, from which a card-board incline led down to the island. A 16 c. p. incandescent lamp was placed near the side to which the inclined plane was attached. After the ants had thoroughly learned the way home, a new incline was attached to the stage on the side opposite the one to which the old incline was still attached. If, after a lapse of five minutes, no ants went down the new incline, conditions were considered right for the test. The light was then transferred to the opposite side of the stage. In each test the halting movements of the ants showed that they were much disturbed. In most cases, some of the ants would finally go down the new incline; and in a few cases, after the lapse of several minutes, all of the ants went down the new incline. The above described experiments with ants working in concert were conducted with several different species.

The individual experiments to test the same thing were conducted with *Formica fusca* var. *subsericea* Say and *Myrmica punctiventris* Rog. The apparatus was arranged in the same manner

as for ants working in concert; but, marked ants were used and only one was allowed to work at a time. In all essential points, the results harmonized with those obtained from ants acting in concert. In most cases, however, after a greater or lesser lapse of time, the ant would usually find its way down the old incline to the nest; and, after a greater lapse of time, find its way back again to the stage.

That the above effect was not due to heat was proven in the following manner. At the right and left of the stage used was placed a tall museum jaw .34 cm. × 16 cm. × 17 cm., filled with distilled water. These heat filters were so adjusted as to have their broad sides parallel to the edges of the stage. At the beginning of the experiment, a 32 c. p. incandescent lamp was placed behind one of these filters (usually behind the one that was near the incline). After the ants had traveled the path long enough to make the trips regularly and rapidly, the lamp was transferred to behind the opposite filter. In every case the workers were much disturbed in the manner stated above. This was repeated with seven different colonies of *Formica fusca*. Since the heat had been excluded, it is evident that the disturbance was the result of some form of light stimulus.

To determine whether the intensity or the direction of light was the determining factor, the experiment was slightly modified. The stage and incline were arranged as before. Sometimes the heat filters were used, but more often they were not. To furnish illumination, four different candle powers (4 c. p., 8 c. p., 16 c. p., 32 c. p.) of incandescent lamps were used, one at a time, in a darkened room. At the beginning of the experiment a lamp of a certain candle-power was placed near the side of the stage to which the incline was attached. After the ants had thoroughly learned the way home a different candle-power was substituted for the first. After the lapse of a few more minutes this lamp was transferred to near the opposite side of the stage. Shortly it was returned to its former position. A few minutes later a different candle-power was substituted for this. This performance was repeated over and over again until each candle-power had been used one or more times.

It was found that substituting a lamp of one candle-power for

a lamp of a different candle-power had no disturbing effect upon the actions of the ants; but that by any marked change in the angular position of the light, no matter what the candle-power, the ants were much disturbed. This forces us to the conclusion that the direction of the rays of light, when present, plays a prominent rôle in the home-going of ants.

ZOÖLOGICAL LABORATORY,
UNIVERSITY OF CHICAGO,
November 10, 1906.

ON THE DEVELOPMENT OF PARASITIC COPEPODS.[1]

PART I.

J. F. McCLENDON.

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The work herein presented was begun at Wood's Hole, 1904, with a view of determining the effects of pressure on the eggs of parasitic copepods. Owing to technical difficulties, three summers have passed with few experimental results, and in consequence the present paper is chiefly of a morphological nature.

I obtained parasitic copepods from fish caught in traps of the Marine Biological Laboratory and the U. S. Fish Commission, at Woods Hole. It was usually necessary to go to the trap to get them fresh, or in the case of some Caligidæ to catch them before they left the fish.

Most of the work was done on *Pandarus sinuatus* Verrill, *Læmargus muricatus* Kröyer (collected by Dr. Conklin), and a new species (text Fig. 3) of the family Dichelesteidæ and of a new

[1] Thesis accepted by the Faculty of the Department of Philosophy of the University of Pennsylvania toward the degree of Doctor of Philosophy.

genus near *Kröyeria* (according to Prof. Charles B. Wilson) which I will call throughout this paper the dichelestid. The eggs of *Pandarus sinuatus* are the most difficult to handle, being very flat and thin and pigmented, but I used them because this is the most common species at Woods Hole. I used the eggs of the following for comparison (arranged according to the classification of Claus):

Caligidæ:

Caligus bonito Wilson (and several undetermined species of *Caligus*).

Caligus rapax Milne Edwards.

Lepioptherius edwardsi Wilson.

Perissopus communis Rathbun.

Nesippus alatus Wilson.

Cecrops latreillii Leach.

Læmargus[1] *muricatus* Kröyer (and an undetermined species of *Læmargus*).

Philorthagoriscus serratus Kröyer.

Pandarus sinuatus Verrill.

Dichelesthiidæ:

Anthosoma crassum Abilguard.

Kröyeria?

Eudactylina nigra Wilson and *E.* sp.?

Lernæidæ:

Penella.

Chondracanthidæ:

Chondracanthus.

Sphyrion?

For the determination of species I am indebted to Prof. Charles Branch Wilson, of Massachusetts State Normal School, Westfield, Mass., who has been very kind in his interest in my work.

After my work was nearly finished I received the material collected at Woods Hole in 1899 by Prof. Edw. Rynearson, of Pittsburgh, with a view of working on this same subject, and which he kindly turned over to me. I wish to express my thanks for this abundance of material, which allowed me to confirm

[1] The word *Læmargus* was applied to a copepod and a shark in the year 1837 and as yet it is disputed which should claim priority.

many points on which my material was scanty and to add some new ones.

I am indebted to Dr. Conklin for constant guidance and assistance besides the general direction of my work, and to Mr. Kribs for the use of Zeiss apochromatic lenses.

Dr. Formad and Dr. Fischelis rendered me invaluable service in translating Russian.

I am under obligations to the Carnegie Institute for a table in the Marine Biological Laboratory, Wood's Hole, 1904, and to the U. S. Fish Commission for a table in the U. S. F. C. Laboratory, Wood's Hole, Mass., 1905–6.

The material was fixed in Flemming's fluid, picro-acetic, or corrosive-sublimate-acetic. The first gave the best fixation but the second was the most convenient. Heidenhain's iron hæmatoxylin followed by various counter-stains, and Hermann's safranin-gentian violet were used most frequently.

II. Historical.

The ground covered in this paper was almost entirely untouched by earlier workers as regards the particular genera treated, and I will consider here only some papers on related forms.

The free living copepods have long been favorite objects for study. Gruber ('79) described their reproductive organs. Weismann and Ischikawa ('88 *a*, *b*) and Ischikawa ('92) used them in studying the question of reduction in number of chromosomes. Haecker ('91, '92 *a*, *b*, '94 *a*, *b*, '95 *a*, *b*, '97, '02) found them favorable objects for study of oögenesis, maturation, reduction, and unsymmetrical mitoses. Later, Rückert ('94, '95 *a*, *b*, *c*) studied oögenesis, maturation, and reduction in these.

Of the parasitic and half parasitic copepods, Heider ('79) notes the spermatogenesis of *Lernanthropus*, and Giesbrecht ('82) the oögenesis of the Notodelphidæ.

Charles Branch Wilson in his monograph on the Caligidæ ('05) describes the anatomy of the reproductive organs and the life history.

III. Anatomy of the Reproductive Organs.

In *Pandarus*, *Caligus* and allied forms the two ovaries lie in the head (Fig. 1) and the two oviducts run backwards to the

genital segment, where they are much convoluted and increase greatly in diameter and each communicates by an opening, the os uteri, with an egg string that trails behind the animal. The ovary is formed of a much convoluted cord of cells in a single linear series, small at the oögonial end and gradually increasing in diameter until it passes into the oviduct. As these cells (oöcytes) grow in the ovary they become much compressed in the direction of the long axis of the cord and the cell boundaries almost completely disappear. Passing into the oviduct, the oöcytes continue to grow and soon their boundaries reappear, and they become more flattened as they grow larger. There are at least two broods in the oviduct at once, caused by the periodic activity of the ovary. The younger brood extends some distance into the genital segment and is sharply marked off from the older brood, which occupies the last coils of the oviduct and consists of oöcytes that have nearly or quite completed their growth. As the oöcytes pass out through the os uteri they are fertilized from the seminal receptacles (Fig. 2, *sr*) and surrounded with secretion from the cement gland (*c.g.*) which forms the wall of the egg string. The eggs are distorted when passing through the thorax, but in most cases regain their symmetrical form. Embryos in the egg strings have their heads turned lateroventrally and their ventral surfaces anteriorly, in relation to the mother. Occasionally one finds an embryo reversed, or partially rotated.

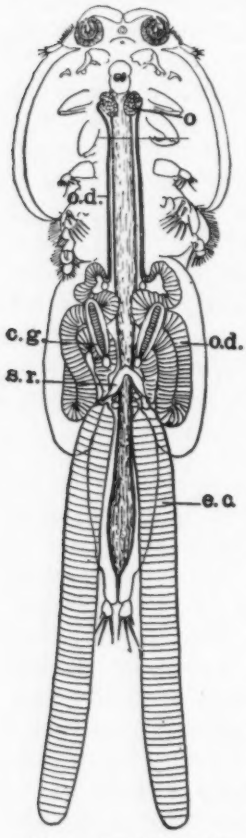


FIG. 1. Female reproductive organs of Caligus bonito. (Drawn by Emerton from Wilson's *Caligidæ*.) *c. g.*, cement gland; *e. c.*, external egg cases; *o*, ovary; *o. d.*, oviduct; *s. r.*, semen receptacle.

In the male the same general arrangement of reproductive organs exists, save that the seminal receptacles are absent and the distal ends of the vasa deferentia are enlarged and become the spermatophore receptacles. The cement glands secrete the material for the walls of the spermatophores.

In the dichelestid (FIG. 3) the genital segment is very much elongated and the oviducts are not convoluted. The ovaries have been carried backward until they lie in the anterior end of the genital

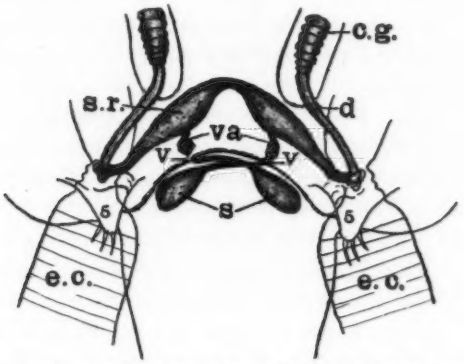


FIG. 2. Semen receptacles and vagina of a female *Lepeophtheirus*. (Partly after Claus.) *c. g.*, cement glands; *d.*, cement gland duct; *e. c.*, egg cases; *s.*, spermatophores; *s. r.*, spermaries; *v.*, vulva; *va.*, vagina; 5, fifth legs. (From Wilson's *Caligidæ*.)

segment and the oviducts pass forward to the anterior end of the thorax, then backward to the attachment of the egg strings. In other respects the description given for *Caligus* will hold for this species. The older brood of oöcytes occupies the posterior two-thirds of the oviduct. When one brood passes into the egg string the distal eggs of the remaining brood have nothing to press against and tend to round up and become distorted. The thickness of the oviduct at its posterior end varies somewhat, depending on the number of eggs produced in one brood, and the size of the eggs. When fewer, the eggs are thicker, and when more numerous, thinner. The egg string is more slender than the posterior portion of the oviduct, and the contained eggs are, therefore, thicker.

In *Læmargus* the egg strings are packed in loops under broad lamellar coverings.

IV. Oögenesis and Maturation.

1. *Oögenesis.*

A. The Dichelestid. — The oögonia (Fig. 1) are very small isodiametrical cells. The nucleus is spherical and contains chromatin granules in a peripheral linin reticulum. Minute nucleoli are also embedded in this reticulum. The cytoplasm stains deeply. I have found but few oögonial mitoses in this species although I have sectioned over a hundred females; the number of chromosomes is the same as in the primary germ cells of the embryo (16) and *twice* the reduced number (8).

The primary oöcyte is readily distinguished from the oögonium by its nucleus being about half the size of the latter (when first formed). The earliest stage I have is the late telophase, in which the chromosomes are drawn together as though at the pole of the spindle, yet the whole mass is surrounded by a nuclear membrane (Fig. 2). The nuclear membrane is therefore formed of the fused linin sheaths of the chromosomes (or from cytoplasm?). This mass is placed excentrically in the nucleus and the individual chromosomes are so pressed together or fused that only their ends sticking out can be distinguished separately, so that it resembles a "synapsis." The cytoplasm stains deeply. The oöcytes are arranged in single linear series as stated above and are pressed and flattened one against the other; the nuclei are close to the free surfaces of the cells, and that edge of an oöcyte containing the nucleus is thicker than the opposite edge. Soon the chromosomes swell and the chromatin becomes

Fig. 3. The *Dichelestid*, ventral view of ♀. The ovaries and oviducts are solid black. Portions of the egg strings are still altached.

dispersed as granules in a peripheral achromatic reticulum, as in the oögonia (Fig. 3). Minute nucleoli appear in this reticulum. The boundaries between the oöcytes become so faint as to be no longer everywhere perceptible. At the same time the cytoplasm begins to increase in volume and the diameter of the egg cord to increase. This process continues until the egg cord passes into the oviduct, and for some time thereafter. When the oöcytes have traversed about a fifth of the oviduct, the cell boundaries reappear and the nucleus migrates to very near the center, there forming an enlargement in the oöcyte (Fig. 4). The reticulum and the three to four nucleoli have grown considerably with the growth of the nucleus. A few yolk spherules and fewer oil globules appear and grow to considerable size. For some time growth of the oöcyte consists almost solely in the addition of yolk spherules and oil globules and this continues until the yolk almost obliterates the cytoplasm and closely surrounds the nucleus. I have seen these spherules and oil globules separated from the protoplasm with a high speed centrifuge (kindness of Dr. Lyon), the former are heavier and the latter lighter than the protoplasm.

B. Læmargus muricatus. — The oögenesis resembles that of the dichelestid. In the oögonial divisions there appear to be sixteen chromosomes, but these are set so close together that I cannot be sure by actual count.

In the growth period the reserve materials, yolk and oil globules, are laid down in ways that show them to be of quite different consistency. The oil globules (dissolved out in sections) appear as minute points that grow in size but always retain a spherical shape. The yolk substances, at least some of them, are laid down as thin discs. A disc receives new substance on its flat surfaces only, and the layers of substances are alternately chromatic and achromatic in staining qualities (when much destained after certain stains). Some of these piles of discs become spherical, others oblong by addition of more discs. The constituent layers do not mix but remain separate until dissoved in the segmenting egg or developing embryo. The oil globules are relatively few in number and large in size. The yolk bodies vary greatly in size, none of them reaching the size of the oil globules.

C. Pandarus sinuatus. — The oöcyte of *Pandarus sinuatus* differs from that of the dichelestid in that a single nucleolus is formed, about equal in bulk to that of the several nucleoli of the latter species together. The oöcyte of the former is much more compressed (thinner) than that of the latter.

2. *Maturation.*

A. Pandarus sinuatus. — At the end of the growth period the nucleus becomes irregular in outline which gives it a shrunken appearance. The nucleolus becomes vacuolated and the nuclear sap intensely staining. The location of chromatin cannot be made out very well, but in thin sections chromatic threads can be seen radiating from the nucleolus. Soon the nuclear sap fades enough to show that the chromatic threads have split (Fig. 6). The nuclear wall is dissolved. Sometimes the vacuoles in the nucleolus increase very much in size and fuse into one. In preparations of the entire oöcyte the chromatic thread can be seen to be divided into eight double chromosomes (Fig. 5). By shortening of these double-rod-shaped chromosomes, ring-shaped chromosomes are formed (Fig. 7). A transverse constriction transforms the diad into a tetrad, that is a ring constricted at four equidistant points, the two opposite constrictions, representing the divisions between the original rods, being deeper than the other two. The spindle when first formed is longer than the shortest diameter of the egg. It is similar to that found in free living copepods, having no polar rays. The dense protoplasm at each end of the spindle (Figs. 7 and 9) may possibly be derived from archoplasm of preceding divisions, and it shows a striking resemblance to the pole plates of the dividing nucleus in Protozoa. The spindle is at first parallel to the flat surfaces of the egg and rotates to an almost vertical position (Fig. 8). The first polar body is very small and is extruded between the egg and its neighbor at about the center of the flat surface that is posterior (in relation to the mother) (Figs. 9 and 10). The second polar spindle is smaller than the first. It is at first parallel to the flat surfaces of the egg and rotates nearly to a perpendicular position under the first polar body (Figs. 9 and 10). I have not seen the second polar body being cut off and

have distinguished only one polar body in later stages, which on account of its size I regard as the first polar body or both polar bodies fused (Fig. 49, *P*). It may be that the second polar body remains in the egg as in *Cyclops* and is not easily distinguishable, or more probably it is extruded immediately under the first polar body and the two being pressed together and becoming very flat, appear as one. The chromosomes in the first polar body do not swell and fuse to form a nucleus.

I have made no observations on the entrance of the sperm. Vide under Polarity of the Egg.

B. Læmargus muricatus (Fig. 18). — Maturation in this species is very similar to the process in *Pandarus*. The disintegration of the single large nucleolus shows many stages which appear to be peculiar to this species. A number of vacuoles (globules of achromatic fluid) appear in it, and these sometimes fuse into a single mass. The chromatic substance does not remain as a continuous peripheral layer but rounds up into four or more masses whose inner surfaces are hemispherical and outer surfaces form portions of the general surface of the nucleolus. In some cases there are two fluids (one colorless and the other staining very faintly) formed within the nucleolus. The colorless fluid forms a large sphere in the center and the faintly staining fluid occupies the periphery and contains spheres of the original chromatic substance of the nucleolus. The chromosomes do not go as far in metamorphosis as in *Pandarus*. In the equatorial plate the chromosomes appear as short double rods (Fig. 18), and if we regard the spireme formed of chromosomes joined end to end and cut into half the usual number of pieces the first maturation division is equational and the second reducing. On the other hand if chromosomes pair in the synapsis stage and lie parallel to form double rods, the first division is reducing and the second equational. The chromosomes cannot be distinguished all through the resting period, and whereas I am inclined toward the view that each rod of the double rod represents a chromosome, I have not sufficient direct evidence to be sure that this is the case.

C. The Dichelestid. — Toward the close of the growth period the nucleus undergoes an enormous change. The nucleoli become

vacuolated and irregular in outline. The reticulum containing chromatin begins to break up and the nuclear sap stains intensely, probably due to the solution of chromatin. The nucleus becomes irregular in outline, probably due to pressure of yolk. From one to three spheres of protoplasm (Fig. 11) are found in some eggs near the wall of the oviduct, which appear to be abnormal structures. With the breaking up of the reticulum, the chromosomes are formed, but the manner in which this occurs is obscured by the intense staining of the nuclear sap. (This process shows better in *Pandarus.*) In order to see any structures in the nucleus it must be de-stained until it is very faint. The nuclear membrane disappears and the karyoplasm increases in volume and soon some of the chromosomes are distinctly double. The nucleoli disappear suddenly. The karyoplasm fades somewhat and becomes filled with alveoles, some of which are very large (Fig. 12). Each chromosome, of which there are eight (Fig. 13), is surrounded by a sphere of homogeneous protoplasm that stains slightly deeper than the surrounding cytoplasm. Each chromosome is a tetrad and often opens out into a ring constricted at four equidistant points. Two opposite constrictions are deeper than the other two and are to be regarded (from comparison with *Læmargus* and *Pandarus*) as the first divisions formed, and probably divisions between whole chromosomes. When seen on edge the tetrad usually appears dumb-bell shaped. There is some variation in the shape of the chromosomes in early prophases (Fig. 12) but in later stages they appear quite uniform in size and shape (Fig. 14). The spheres of homogeneous protoplasm surrounding the chromosomes fuse into one mass. A colorless area appears around each chromosome (Fig. 13) which makes it appear as though each chromosome was enclosed in a linin sac.

These linin "sacs" are each drawn out in the form of a spindle, and these spindles, lying parallel, form the first maturation spindle. Spindle fibers develop (Figs. 13 and 14) and some of them become attached to the chromosomes. The spindle is elongated, and at each pole the protoplasm stains more intensely resembling the pole plate in protozoön mitosis (Fig. 16). The paler protoplasm forms a sphere around each pole of the spindle,

(Fig. 16). From these spheres radiate strands of protoplasm simulating astral rays, but not so dense. The spindle is formed parallel to the flat surfaces of the egg and then begins to rotate to a perpendicular position, at the same time shortening (Fig. 17). In this state it remains until fertilization, and further than this I have not followed it. The behavior of the linin sheath of the chromosome is very peculiar, and it seems to be more conspicuous than in any other instance I know of. The spindle, in general appearance, resembles the first cleavage spindle of *Cyclops* (Häcker).

V. Spermatogenesis.

Læmargus muricatus. (*Preliminary Notice.*)

The spermatogonia are small cells with comparatively large spherical nuclei, and are arranged in single linear series. The nucleus contains chromatin granules in a peripherical linin reticulum. When preparing for mitosis these granules become arranged into looped rows, but I have not ascertained whether these rows form a continuous spireme or not. There is a single large nucleolus. In the last spermatogonic divisions there are 16 chromosomes (twice the number of the spermatocytic divisions). The preliminary spermatocytes are half the size of the spermatogonia when first formed, and are arranged in single linear series. Whether there is a single chain or cord of cells in the testes as in the ovaries, I cannot tell, owing to the many convolutions, but think there are at least several such chains or cords. The regular growth of these causes the testis to be divided into zones. During the growth period the cells grow to the size of the spermatogonia and cannot be distinguished from them save by their position in the testis (growth zone). When preparing for division the cells lose their linear arrangement; the chromatin forms eight double rods which lie close together (synapsis stage). Each double rod becomes ring shaped and the rings contract until only a small lumen is left. An additional pair of constrictions converts the ring into a tetrad. The division forms two secondary spermatocytes, each with eight diads, and another division immediately following forms four spermatids, each with eight chromosomes. The spermatids are grouped in fours. In

some of these groups, each of the cells becomes filled with an achromatic substance which presses the chromatin and protoplasm against the cell wall. This substance increases greatly in quantity and becomes more and more like yolk (called Austreibestoff by C. Heider, '79). In very rare cases in some very young spermatids I have seen a small achromatic sphere in the cytoplasm and have found a still smaller sphere in the cytoplasm of some primary spermatocytes. On the other hand the substance in question at first appears to lie within the nucleus and is closely surrounded by chromatin. A plausible explanation might be that the small globule seen in some very young spermatids moved up to and indented the nucleus, there growing until it became larger than the original nucleus yet I have been unable to find steps in such a process, in fact I believe the substance arises in the nucleus.* The cells containing these spheres nourish the spermatozoa, and may be called nutritive cells.

Going back to the spermatids, many groups degenerate, becoming much shrunken, while those that will form spermatozoa collect into larger groups, the cells of which begin to elongate radially. As the spermatids become longer they come to lie nearly parallel. The nucleus elongates into a spindle shaped deeply staining fiber, covered by a thin layer of achromatic substance. The remaining protoplasm fuses with that of adjacent spermatozoa and forms a mass in the center of the group. These groups sometimes lie with one end against a nutritive cell, and when the groups break up the spermatozoa collect around nutritive cells until the cytoplasm of the latter disintegrates and the chromatin collects into rounded masses that float about, leaving only a sphere of a yolk-like substance, the achromatic substance, which has now developed an affinity for plasma stains. As the elements pass down the vas deferens the nutritive spheres lie near its walls and on reaching the spermatophore the nutritive spheres (Austreibekörperchen) form a layer several spheres deep, and become pressed together into polyhedrons. Most of the chromatic spherules form a layer within the nutritive layer but some chromatic spherules caught between the nutritive spheres,

*Compare formation of "Glanzkörper" from the plasmosomes of Pelomyxa: Goldschmidt, Arch. f. Protistenkunde 5, p. 130.

become elongate curved bodies that remain for a long time. The spermatozoa fill the center of the vas deferens and lie parallel to one another. In the spermatophores they extend radially from the nutritive walls. In spermatophores that have been attached to the female a long time, the nutritive material has disappeared, leaving a mass resembling evacuated cell walls.

The same description of the spermatogenesis holds in general for *Pandarus sinuatus*, though there are many minor differences. As regards the behavior of these nutritive bodies I have observed one similar instance, in *Peripatus*, but in *Peripatus* the nutritive bodies are nuclei of degenerating cells. In these copepods the nutritive body appears to form in or in close relation to the nucleus, but too little is known both of the nutritive bodies in *Peripatus* and copepods to suppose them genetically related.

VI. Polarity of the Egg.

The fertilized egg and embryo in all cases in which the egg string extends straight behind the animal and contains a single linear series of eggs, is definitely oriented in relation to the mother. The animal pole of the egg is posterior and the first protoplasmic cell and resulting head end of the embryo is latero-ventral. The chief axis of the egg is manifested in the ovary in the flattening of the egg (being the shortest axis). In any stage in which the primary oöcyte is considerably thicker than the diameter of the nucleus, the latter lies nearer the animal pole. The head end of the embryo coincides with the region in which the first protoplasmic cell is formed, and this is probably determined by the point of entrance of the sperm. The seminal receptical opens into the oviduct by a small orifice which would lead sperm to the egg at or near the position of the future head of the embryo. Cases of rotation of the long axis of the embryo, which sometimes occur, might be due to the spermatozoön getting around the egg before entering.

The first polar body is extruded in a slightly eccentric position on that flat side of the egg directed toward the free end of the egg string. Thus the chief axis of the egg does not exactly coincide with the shortest axis, but is a little inclined toward the anterior end, yet not enough to cause the first polar body to lie

in the first cleavage furrow. This is probably due to the great inequality in the first cleavage. Furthermore, the blastopore does not close at a point diametrically opposite to the first polar body, but considerably posterior to such a point. From a study of the literature on gastrulation in crustacea, I am led to believe that the blastopore in the majority of cases in this group closes posterior to the vegetal pole and such a character would be accentuated by flattening of the egg. As the eggs occur in single linear series in an egg string surrounded on all sides by sea water, there is probably nothing in the surroundings that could determine the axes of the egg, and we should regard them as probably determined by the structure of the protoplasm.

VII. Summary.

1. In the maturation of the eggs of parasitic copepods the behavior of the chromosomes in regard to the question of *reduction* is very similar to the same process in the free living copepods, yet I differ from Haecker as to the reducing division, considering the first maturation division most probably to be the reducing division.

2. In the spermatogenesis only a small proportion of the spermatids become spermatozoa. Many spermatids degenerate, others become metamorphosed into peculiar nutritive cells. The protoplasm of the nutritive cells degenerates leaving only a sphere of deutoplasm" "Austreibekörperchen," which C. Heider ('79) thought was a glandular secretion.

IX. Abbreviations.

n = Nucleolus.
p = First polar body.
s = Sphere of protoplasm, probably an abnormal structure
x = Darkly staining cell in yolk.
y = Yolk spherule.

Explanation of Plate I.

(*Figs. 1–14, The Dichelestid.*)

Fig. 1. An Oögonium.

Fig. 2. Three consecutive primary oöcytes in the synapsis stage. Optical section of the cord of ovarian cells.

Fig. 3. Early growth stage of same, partial disappearance of cell walls.

Fig. 4. Middle portion of oöcyte containing the nucleus, from longitudinal section of oviduct. Commencement of formation of yolk spherules (*y*). Two nucleoli are seen (*n*).

(*Figs. 5–10, Pandarus sinuatus Verrill.*)

Fig. 5. A little later stage. Nucleus viewed from the animal pole. Two of the eight double rods, or diads, are joined together end to end (to the right), so that it is difficult to distinguish them separately.

Fig. 6. The same in a section through the short axis containing six of the diads. Above at *n*, the nucleolus of another egg is represented to show a later stage in the disintegration of the nucleolus.

Fig. 7. The first maturation spindle.

Fig. 8. Later stage of same, in which it has rotated through almost a right angle and is pushing through the egg membrane to extrude the first polar body.

Fig. 9. Late metaphase of the second polar spindle. The first polar body is represented at *p*. (Constructed from two sections of the series.)

Fig. 10. The same stage from a surface preparation of the egg. The wall of the oviduct was torn off and the eggs allowed to separate in sea water (two hours). In separation the first polar body is pulled out into a stalked structure.

(*Figs. 11–17, The Dichelestid.*)

Fig. 11. Less magnified figure of prophase showing sphere of cytoplasm (*s*) near periphery of egg.

Fig. 12. A later stage. The nuclear sap has faded, it is vacuolated, and around each chromosome stains darker (*f*) than elsewhere. The two chromosomes that are stippled are out of focus. The chromosomes are ring-shaped tetrads.

Fig. 13. The chromosomes are each enclosed in a linin sac, and these sacs have begun to elongate.

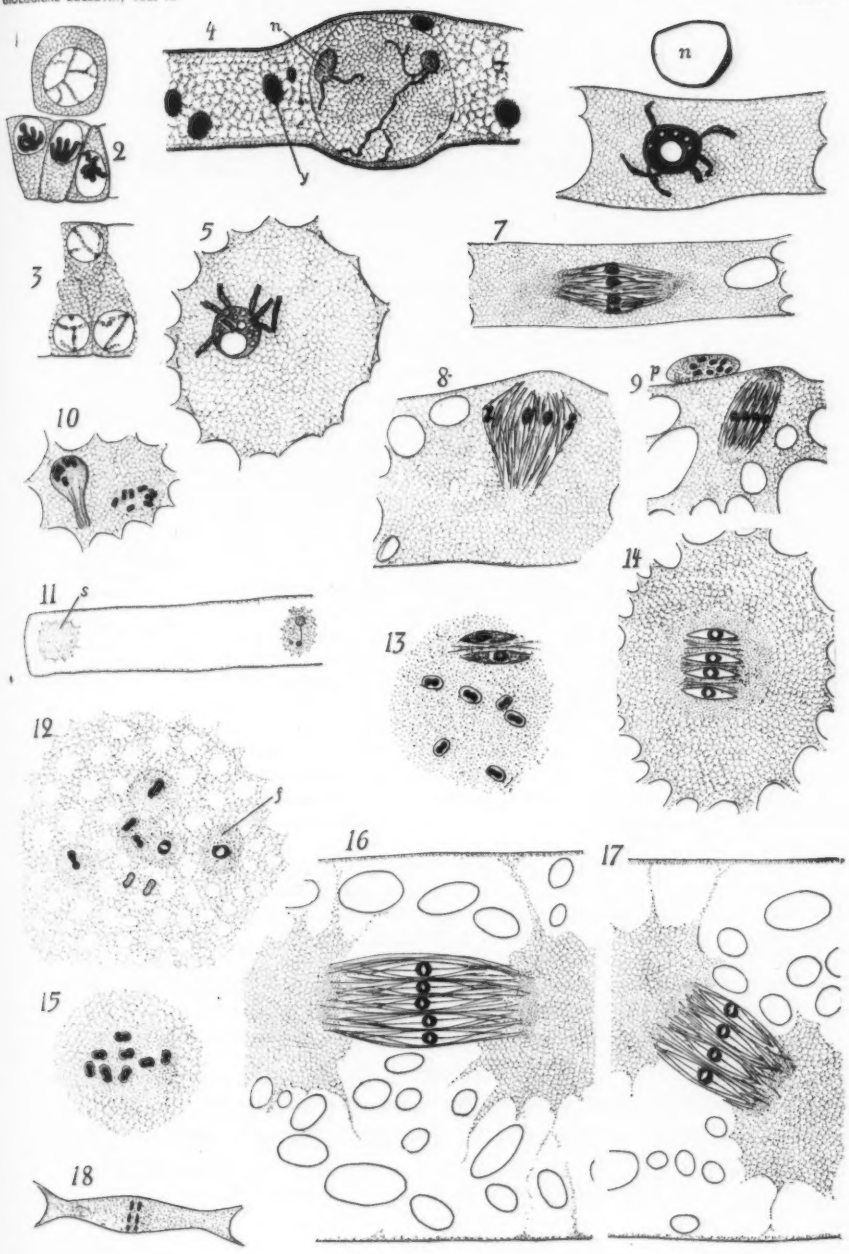
Fig. 14. The same or a little later stage. The elongated linin sacs lie parallel, with spindle fibers developed between them. The incipient spindle is formed in dense (dark) protoplasm while this latter is surrounded by looser (paler) protoplasm.

Fig. 15. Equatorial plate, same stage as 14.

Fig. 16. The spindle has elongated perpendicular to the short axis of the egg. The dense protoplasm forms the two poles of the spindle. While the loose protoplasm forms astrosphere-like structures enclosing the poles of the spindle.

Fig. 17. The spindle is half rotated toward the short axis of the egg and has considerably shortened in doing so.

Fig. 18. *Læmargus muricatus* Kröyer. Central portion of the egg containing the first maturation spindle. Metaphase. The spindle fibers do not show in this preparation. Three of the eight chromosomes are in focus, one being seen from the end and appearing smaller than the others.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